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Preparation of yeast membranes

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An abbreviated version of this protocol was published in Science Advances in May 2021

Mucosal immunity–mediated modulation of the gut microbiome by oral delivery of probiotics into Peyer's patches

DOI: 10.1126/sciadv.abf0677

Detailed protocol

Materials and Reagents

1. Baker's yeast: (Lesaffre, catalog number: GB/T 20886)
2. Sodium hydroxide: (Greagent, catalog number: G19852A)
3. Hydrochloric Acid: (Sinopharm, catalog number: 10011018)
4. Aceton: (Sinopharm, catalog number: 10000418)
5. Isopropyl alcohol: (Sangon Biotech, catalog number: A600918)
6. Glass beads: (Solarbio, catalog number: G8080)
7. 500 ml Conical flask: (Titan, catalog number: 02053542)
8. 50 ml Syringe: (Titan, catalog number: 02026695)
9. 50 ml centrifuge tubes: (Titan, catalog number: 02046667)
10. 1.5 ml centrifuge tubes: (Titan, catalog number: 02036208)
11. 0.6 ml centrifuge tubes: (Titan, catalog number: 02036207)
12. Aluminum foil: (Titan, catalog number: 02038479)

Equipment:

1. Water bath with magnetic stirring: (Yuhua, type: ZNCL-G)
2. Vortex shaker: (IKA, type: type: SI-0236)
3. Centrifuge: (Eppendorf, type: 5810R and 5424R)

Procedure

1. Preheat the water bath to 80 ℃.
2. 20 g Baker's yeast is suspended with 300 ml of 1 M sodium hydroxide in a 500 ml conical flask. Cover the conical flask with aluminum foil.
3. Incubate the suspension at 80 °C for 1.5 hours with magnetic stirring and then transfer the suspension with the same volume into 50 ml tubes and centrifuge the suspension at 3,000 rpm for 10 minutes at room temperature.
4. Pour out the supernatant and rinse the pellet twice with deionized water.
5. Resuspend the pellet with 300 ml of the prepared hydrochloric acid aqueous solution (pH=4) and transfer the suspension into a 500 ml conical flask and incubate the suspension at 60 °C for 1.5 hours under magnetic stirring.
6. Centrifuge the suspension at 3,000 rpm for 10 minutes at room temperature.
7. Pour out the supernatant and rinse the pellet twice with deionized water.
8. After centrifuging at 3,000 rpm for 10 minutes at room temperature, resuspend the pellet with isopropyl alcohol and centrifugate again at the same condition. Repeat this procedure 3 times.
9. After centrifuging at 3,000 rpm for 10 minutes at room temperature, resuspend the pellet with acetone and centrifugate again at the same condition. Repeat this procedure 1 time.
10. Centrifugate the suspension and pour out the supernatant. Dry the pellet in the fume cupboard at room temperature for around 3 hours and store the sample at 20 ℃.
11. To prepared yeast membrane for bacteria coating, mix 500 ul of the pellet (10 mg/ml in deionized water) with the same volume of glass beads (300-500 nm) in a 1.5 ml tube and shake the mixture at 3,200 rpm for 2 hours at room temperature (30 minutes for one circle with 5 minutes stop at each circle).
12. Centrifuge the sample shortly and collect the supernatant without glass beads (yeast membranes) and transfer the glass beads to a 0.6 ml tube and put the tube to a 1.5 ml tube after piercing a small hole in the bottom of the 0.6 ml tube with a needle of 50 ml syringe.
13. Collect the liquid (yeast membranes) after centrifuging the tube at 12,000 rpm for 15 minutes.
14. The yeast membranes are prepared when they will be used.

Related files

Protocol of YMs preparation.pdf

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. lin, s. , Mukherjee, S. , Li, J. , Hou, W. , Pan, C. and Liu, J. (2021). Preparation of yeast membranes. Bio-protocol Preprint. bio-protocol.org/prep1101.
2. Lin, S., Mukherjee, S., Li, J., Hou, W., Pan, C. and Liu, J.(2021). Mucosal immunity–mediated modulation of the gut microbiome by oral delivery of probiotics into Peyer's patches. Science Advances 7(20). DOI: 10.1126/sciadv.abf0677